Effect of sodium butyrate on growth performance and response to lipopolysaccharide in weanling pigs

T. E. Weber and B. J. Kerr

J Anim Sci 2008.86:442-450.
doi: 10.2527/jas.2007-0499 originally published online Nov 12, 2007;

Effect of sodium butyrate on growth performance and response to lipopolysaccharide in weanling pigs[1]

T. E. Weber[2] and B. J. Kerr

USDA-ARS, National Swine Research and Information Center, Ames, IA, 50011-3310

ABSTRACT: Two experiments were conducted to determine the effects of dietary sodium butyrate on growth performance and response to *Escherichia coli* lipopolysaccharide (LPS) in weanling pigs. In a 28-d experiment, 180 pigs (initial BW 6.3 kg) were fed 0, 0.05, 0.1, 0.2, or 0.4% sodium butyrate, or 110 mg/kg of dietary tylosin. There was no effect of dietary sodium butyrate or tylosin on overall G:F, but there was a linear trend ($P < 0.07$) toward decreased ADFI and ADG as levels of sodium butyrate increased. In a second 28-d experiment, 108 pigs (initial BW 6.3 kg) were assigned to 1 of 3 dietary treatments: 1) no antibiotics, 2) 0.2% sodium butyrate, or 3) 55 mg/kg of carbadox. On d 14, a subset of pigs from the no-antibiotic and butyrate treatment groups was challenged with *E. coli* LPS or injected with sterile saline in a 2 × 2 factorial arrangement (±LPS challenge; ±dietary butyrate; n = 6 pigs/treatment group). Four hours after LPS challenge, blood samples were obtained, and samples of LM, liver, and ileum were collected for gene expression analysis. Serum samples were analyzed for IL-6, tumor necrosis factor α (TNFα), α_1-acid glycoprotein, cortisol, IGF-I, insulin, and metabolites. The relative abundance of tissue cytokine and IGF-I mRNA was measured by real-time PCR. Feeding diets containing sodium butyrate or carbadox did not alter ADG or ADFI compared with pigs fed the control diet. From d 0 to 14, pigs fed diets containing 0.2% sodium butyrate had decreased ($P < 0.05$) ADG and tended ($P < 0.06$) to have decreased G:F compared with animals fed diets containing carbadox. Challenge with LPS increased ($P < 0.05$) serum cytokines and cortisol and decreased ($P < 0.05$) serum glucose and triglycerides. Injection with LPS increased ($P < 0.05$) the relative abundance of hepatic IL-6 and TNFα mRNA, increased ($P < 0.05$) LM TNFα mRNA content, and decreased ($P < 0.05$) IGF-I mRNA in LM. For serum cortisol, there was an interaction ($P < 0.05$) between dietary butyrate and LPS. The increase in serum cortisol attributable to LPS was greater ($P < 0.05$) in pigs fed butyrate than in pigs fed the control diet. There tended ($P < 0.10$) to be an interaction between LPS and diet and for butyrate to increase the relative abundance of IL-6 mRNA in LM. Carbadox did not alter cytokine or IGF-I mRNA or serum metabolites, but did decrease ($P < 0.05$) serum TNFα. These data indicate that dietary sodium butyrate does not enhance growth performance, but may regulate the response to inflammatory stimuli in weanling pigs.

Key words: butyrate, growth performance, lipopolysaccharide, weanling pig

J. Anim. Sci. 2008. 86:442–450
doi:10.2527/jas.2007-0499

INTRODUCTION

Butyrate, a short-chain fatty acid produced by the bacterial fermentation of dietary fiber, has been found to influence the inflammatory response. Sodium butyrate directly decreases production of inflammatory cytokines by macrophages in response to *Escherichia coli* lipopolysaccharide (**LPS**; Rodriguez-Cabezas et al., 2003; Fukae et al., 2005). Furthermore, sodium butyrate induces histone acetylation in vitro (Li and Elsasser, 2005) and in vivo (Schroeder et al., 2007), and oral administration of a histone deacetylase inhibitor to mice before challenge with LPS decreases systemic production of proinflammatory cytokines (Leoni et al., 2005). Challenge with LPS induces catabolic events in muscle tissue, including a downregulation of IGF-I expression (Spurlock et al., 1998). Previous studies have found that supplementation with sodium butyrate increases growth performance and intestinal integrity

[1]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable. The authors acknowledge the National Pork Board (Des Moines, IA) for financial support and INVE Nutri-Ad (Kasterlee, Belgium) for providing the sodium butyrate used in these studies. The authors thank Shari Steadham and Melissa Weber for assistance with tissue sample collection.

[2]Corresponding author: Tom.weber@ars.usda.gov

Received August 6, 2007.
Accepted October 29, 2007.

(Kotunia et al., 2004) in piglets fed a liquid diet. In addition, nursery pigs fed sodium butyrate have increased feed efficiency (Manzanilla et al., 2006). Broilers fed a diet containing 0.2% butyric acid had increased ADG and breast muscle yield (Leeson et al., 2005). Regulation of growth by butyrate may be mediated in part by its impact on the inflammatory response. However, to date there are no published data regarding the effects of feeding sodium butyrate to pigs on their response to inflammatory stimuli.

The objectives of the current study were to determine the growth response of weanling pigs to various dietary levels of sodium butyrate and to compare the response to subtherapeutic levels of antibiotics. We also sought to determine whether feeding sodium butyrate would alter the inflammatory and metabolic responses to LPS in weanling pigs. Last, effects of the dietary antibiotic carbadox on steady-state markers of inflammation and metabolites were also measured.

MATERIALS AND METHODS

Animals, Experimental Design, and Sample Collection

All animal care and handling procedures used in these studies were reviewed and approved by the Iowa State University Animal Care and Use Committee.

For the first experiment, 180 crossbred pigs, (C22 × 337, Pig Improvement Company, Franklin, KY; initially 6.3 ± 0.4 kg of BW) were blocked by ancestry, sex, and initial BW and randomly assigned to 1 of 6 treatments: 1) no antibiotics; 2) 0.05% sodium butyrate (Adimix CP, INVE Nutri-Ad, Kasterlee, Belgium); 3) 0.1% sodium butyrate; 4) 0.2% sodium butyrate; 5) 0.4% sodium butyrate; or 6) 110 mg/kg of tylosin (Tylan, Elanco Animal Health, Greenfield, IN). Pigs were reared in groups of 5 pigs/pen, with 6 pens per dietary treatment. Experimental diets were fed in 2 phases, each lasting 2 wk, for a period of 4 wk postweaning (Table 1). Body weights and feed intake were measured every 2 wk. Pigs were allowed ad libitum access to feed and water. All diets were formulated to meet or exceed NRC (1998) requirements. Sodium butyrate and tylosin premixes were added to the diets at the expense of corn.

In a second experiment to evaluate the response of pigs fed sodium butyrate to LPS, and to examine the growth response to sodium butyrate or carbadox, 108 crossbred pigs of similar genetic background as those in Exp. 1 were blocked by sex, litter of origin, and initial BW (6.3 ± 0.4 kg), and were assigned to 1 of 3 dietary treatments: 1) no antibiotics, 2) sodium butyrate at 0.2%, or 3) 55.0 mg/kg of carbadox (Phibro Animal Health, Ridgefield Park, NJ). The 0.2% sodium butyrate level was chosen for this experiment to prevent a depression in ADFI that could confound the inflammatory response, yet we wished to maximize butyrate intake. Results from Exp. 1 suggested that overall ADFI did not decrease until butyrate levels approached 0.4%. In addition, studies in broilers (Leeson et al., 2005) found that feeding 0.2% butyric acid increased breast muscle yield. Experimental diets were fed in 2 phases for a period of 4 wk postweaning. Body weights and feed intake were measured every 2 wk. Pigs were reared in groups of 6 pigs/pen, and there were initially 6 pens per treatment group.

At the completion of the first 2-wk dietary phase, a subset of the pigs (1 pen, n = 6 pigs) from each of the control and the sodium butyrate-fed groups were injected i.m. with *E. coli* LPS (25 μg/kg of BW, *E. coli* serotype O55:B5, Sigma Chemical Co., St. Louis, MO) or with sterile saline in a 2 × 2 factorial arrangement (±LPS challenge; ±dietary butyrate; n = 6 pigs/treatment group). A subset (n = 6 pigs) of the pigs fed carbadox-containing diets were also injected with sterile saline solution and slaughtered for tissue collection. The subset of pigs used for the LPS injection experiment were deprived of feed for 4 h before initiation of the LPS injection and for the following 4 h to avoid confounding the results because of differences in feed intake. Four hours after the LPS or saline injection, blood samples for serum were obtained from the vena cava, and the pigs were killed by penetrating captive bolt. A sampling time of 4 h was chosen because a previous study found that LPS decreased the relative abundance of IGF-I mRNA in LM at 4 h after LPS challenge (Spurlock et al., 1998). Likewise, elevated levels of tissue (Brix-Christensen et al., 2005) and circulating (Wright et al., 2000) cytokines have been found in pigs at 4 h after LPS injection. Samples of LM, liver, and ileum tissues were collected, rinsed with sterile PBS, snap-frozen in liquid nitrogen, and stored at –80°C pending gene expression analysis. A sample of whole ileal tissue was collected 15 cm cranial to the ileal-cecal junction. Serum samples were stored at –80°C until analyzed for cytokines and metabolites.

Serum Cytokines, Cortisol, and Metabolites

Serum concentrations of IL-6 and tumor necrosis factor α (**TNFα**) were measured by using porcine-specific ELISA kits (R & D Systems, Minneapolis, MN), and measurements were conducted according to the manufacturer's recommendations. The limit of detection for the IL-6 ELISA was 10.0 pg/mL, and the intra- and interassay CV were 2.9 and 8.5%, respectively. The TNFα ELISA had a limit of detection of 3.7 pg/mL and intra- and interassay CV of 4.6 and 8.4%, respectively. When the concentrations of IL-6 were below the limit of detection for the ELISA, the limit of detection was used as the concentration for data analysis. Serum α_1-acid glycoprotein (**AGP**) was measured by using a porcine-specific radial immunodiffusion kit (Cardiotech Services Inc., Louisville, KY). The intra- and interassay CV were 4%, and the range of detection was 50 to 1,500 μg/mL. Serum IGF-I concentrations were determined by using a commercially available kit (Active IGF-I ELISA, Diagnostic Systems Laboratories Inc., Webster,

Table 1. Composition of experimental diets (as-fed basis)

Item	Phase 1 (wk 1 through 2)	Phase 2 (wk 3 through 4)
Ingredient, %		
Corn	43.88 to 44.41	56.87 to 57.14
Soybean meal (47.3% CP)	25.10	23.39
Dried whey	20.00	10.00
Select menhaden fish meal	4.00	4.00
Vegetable oil	2.50	2.10
Dicalcium phosphate	1.22	1.72
Spray-dried porcine plasma	1.00	—
Limestone	0.40	0.34
Salt	0.35	0.35
Vitamin and trace mineral premix[1]	0.65	0.65
DL-Met	0.13	0.07
L-Lys·HCl	0.18	0.18
L-Thr	0.06	0.05
Sodium butyrate premix[2]	0 to 0.47	0 to 0.47
Antibiotic[3]	0 to 0.25	0 to 0.25
Calculated analysis		
ME, kcal/kg	3,375	3,375
CP, %	21.20	19.35
Lys, %	1.44	1.27
Ca, %	0.90	0.90
P available, %	0.55	0.55

[1]Provided per kg of diet: vitamin A, 11,023 IU; vitamin D_3, 2,756 IU; vitamin E, 55 IU; vitamin B_{12}, 55.0 μg; riboflavin, 16.535 mg; pantothenic acid, 44.1 mg; niacin, 82.7 mg; Zn, 150 mg; Fe, 175 mg; Mn, 60 mg; Cu, 17.5 mg; I, 2 mg; and Se, 0.3 mg.

[2]Pigs were fed diets containing 0.00 to 0.47% of a product containing 85% sodium butyrate to achieve final levels of 0, 0.05, 0.1, 0.2, or 0.4% sodium butyrate. A level of 0.2% sodium butyrate was used to evaluate the effect of sodium butyrate on the response to lipopolysaccharide (LPS).

[3]Provided per kg of complete diet: 110 mg of tylosin for the butyrate dose study or 55.0 mg of carbadox for the LPS challenge study.

TX). Plasma samples were pretreated with the solutions provided with the kit to separate IGF-I from binding proteins. The samples were run in duplicate using the manufacturer's protocol. The assay was validated for porcine plasma by spiking a pooled porcine plasma sample with known quantities of standard and by serial dilution of the pooled plasma sample. Based on 2 assays, the intra- and interassay CV were less than 9%. The percentage recovery from pooled porcine plasma was 99%, and the assay sensitivity was 0.03 ng/mL. Serum cortisol was determined by using a commercially available kit (Active Cortisol EIA, Diagnostic Systems Laboratories Inc.) that had been validated previously for porcine serum (Weber and Spurlock, 2004). The cortisol ELISA had a limit of detection of 1 ng/mL and intra- and interassay CV of less than 12%. Serum triglycerides were quantified by using an enzymatic kit purchased from Pointe Scientific Inc. (Lincoln Park, MI). Triglycerides were measured by using a lipase to convert the triglycerides to glycerol and FFA. Glycerol kinase and glycerophosphate oxidase were then used to derive H_2O_2. A peroxidase enzyme was then used to derive a red color product that was measured at 540 nm. The intra- and interassay CV for the triglyceride assay were 1.0 and 2.9%, respectively. Glucose concentrations were determined by using an enzymatic kit (Sigma Chemical Co.) based on hexokinase activity. Serum urea nitrogen was determined by using previously described methods for nursery pigs (Kerr et al., 2004). The glucose and serum urea nitrogen assays had intra- and inter assay CV of less than 10%. Concentrations of NEFA were measured by using a commercially available kit (NEFA C, Wako Chemicals Inc., Richmond, VA) and the instructions included with the kit. Intra- and interassay CV for the NEFA assay were 1.4 and 2.7%, respectively. Serum insulin concentrations were determined by using a porcine-specific insulin ELISA kit (Alpco, Windham, NH). The insulin ELISA had a range of detection of 0.02 to 1.5 ng/mL and intra- and interassay CV of less than 10%.

RNA Isolation and Real-Time Reverse Transcription PCR

Total RNA was isolated from LM, liver, and ileum tissue samples by using Trizol (Invitrogen Inc., Carlsbad, CA) reagent according to the manufacturer's protocol, and the RNA pellets were resuspended in nuclease-free water. To eliminate possible genomic DNA contamination, the RNA samples were treated with a DNase I kit (DNA-free, Ambion Inc., Austin, TX) per the manufacturer's instructions. Total RNA was quantified by measuring the absorbance at 260 nm with a NanoDrop ND-100 spectrophotometer (NanoDrop Technologies, Rockland, DE), and the purity was assessed by determining the ratio of the absorbance at 260 and 280

Table 2. Porcine-specific primers used for real-time PCR

Gene	Symbol	Primer sequences[1] (5′ → 3′)	Product size, bp	Accession no.
IGF-I	IGF1	(S) GCCTCAGGGCTCAATTCATA (AS) GGTGCCTGACAAGGACGTAT	137	X64400
IL-6	IL6	(S) GCCACCTCAGACAAAATGCT (AS) TCTGCCAGTACCTCCTTGCT	143	NM_214399
TNFα[2]	TNFA	(S) CCCAAGGACTCAGATCATCG (AS) ATACCCACTCTGCCATTGGA	101	X57321
Cyclophilin A	PPIA	(S) GCGTCTCCTTCGAGCTGTT (AS) CCATTATGGCGTGTGAAGTC	160	AY008846

[1]S = sense primer; AS = antisense primer.
[2]Tumor necrosis factor α.

nm (NanoDrop). All samples had 260/280 nm ratios above 1.8. Additionally, the integrity of the RNA preparations was verified by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 1.2% agarose gels (E-gel, Invitrogen Inc.). A good preparation was indicated by the presence of 28S and 18S bands that were not smeared, and by the 28S band being stained with a greater intensity than the 18S band. Total RNA (1 μg) was reverse transcribed by using a commercially available cDNA synthesis kit (iScript, Bio-Rad Laboratories, Hercules, CA). The iScript kit uses a blend of oligo (dT) and random hexamer primers for cDNA synthesis, and the reverse transcription is RNAse H+ to ensure removal of the RNA template.

Real-time PCR detection of the mRNA was conducted by using the SYBR Green assay. Primers used for real-time PCR are presented in Table 2. Amplification was carried out in a total volume of 25 μL containing 1× iQ SYBR Green Supermix (Bio-Rad), forward and reverse primers (0.1 μg/μL), and 1 μL of the 20-μL cDNA reaction. After an initial 5-min denaturation step at 95°C, the reactions were cycled 40 times under the following parameters: 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Optical detection was carried out at 72°C. At the end of the PCR, melt curve analysis was conducted to validate the specificity of the primers. Thermal cycling conditions and real-time detection were conducted by using an Opticon real-time PCR detection system (Bio-Rad Laboratories). A nontemplate control was run with every assay, and all determinations were performed in duplicate. The presence of a single PCR product of the correct size for each primer set was verified by visualizing the PCR products via electrophoresis on 1% agarose gels stained with ethidium bromide. External cDNA standards were constructed by cloning the corresponding reverse transcription PCR product into a pCR 4-TOPO vector (Invitrogen Inc.), and the resultant plasmids were sequenced for verification. The abundance of each gene product was calculated by regressing against the standard curve generated in the same reaction with their respective plasmids. The RNA abundance values for each sample were normalized to cyclophilin A. The mRNA expression of cyclophilin A was not affected by dietary treatment or LPS injection in any of the tissues.

Statistical Analysis

Data were analyzed as a randomized complete block design by using the GLM procedure (SAS Inst. Inc., Cary, NC). To determine effects of increasing levels of dietary sodium butyrate or including dietary antibiotic on growth performance and feed intake, the data were analyzed by single-factor ANOVA with the GLM procedure of SAS. The model included dietary treatment, and the residual mean square error was used as the error term. Linear and quadratic contrasts were also performed to determine the dose-response to sodium butyrate. Pen served as the experimental unit and differences were considered statistically significant at $P < 0.05$. For the second experiment, in which the effects of 0.2% sodium butyrate or carbadox on growth performance and feed intake were analyzed, the data were analyzed by single-factor ANOVA to test the effect of dietary treatment. To determine effects of dietary butyrate on the response to LPS, the serum and gene expression data were analyzed as a 2 × 2 factorial arrangement (±LPS challenge; ±dietary butyrate) by using the GLM procedure of SAS. The model included the effects of dietary butyrate, LPS treatment, and the 2-way interaction. To analyze the effects of dietary butyrate or antibiotic on constitutive cytokine and IGF-I expression in non-LPS-challenged pigs, serum and gene expression data from the 3 means of interest (no antibiotics, no LPS; butyrate, no LPS; antibiotics, no LPS) were analyzed as a separate, single-factor ANOVA. If a statistically significant treatment effect ($P < 0.05$) was found, the treatment means were separated by using the Student-Newman-Keuls multiple-range test. Trends were discussed and treatment means were separated when the ANOVA indicated $P < 0.10$. For gene expression and serum data, individual pig served as the experimental unit. The residual mean square error term was used to test all main effects and interactions.

Table 3. Effects of increasing levels of sodium butyrate (NaB) or tylosin on the growth performance of weanling pigs (Exp. 1)

	Treatment[1]								Contrast *P*-value	
Item	0%	0.05%	0.1%	0.2%	0.4%	Tylosin	SE	Model *P*-value[2]	Linear	Quadratic
Initial BW, kg	6.3	6.3	6.3	6.2	6.2	6.3	0.4	0.99	0.85	0.98
Final BW, kg	13.0	13.4	13.0	13.0	12.2	13.1	0.6	0.87	0.15	0.28
ADG, kg										
Phase 1	0.142	0.149	0.143	0.132	0.140	0.121	0.009	0.29	0.49	0.60
Phase 2	0.391	0.414	0.392	0.412	0.337	0.430	0.033	0.44	0.08	0.09
Week 1 to 4	0.257	0.271	0.258	0.261	0.231	0.264	0.017	0.68	0.07	0.13
ADFI, kg										
Phase 1	0.189	0.203	0.198	0.176	0.188	0.184	0.009	0.39	0.33	0.51
Phase 2	0.539	0.574	0.542	0.531	0.487	0.560	0.036	0.64	0.05	0.13
Week 1 to 4	0.350	0.374	0.357	0.340	0.326	0.358	0.020	0.65	0.06	0.16
G:F										
Phase 1	0.754	0.733	0.725	0.746	0.743	0.652	0.032	0.27	0.96	0.95
Phase 2	0.723	0.724	0.720	0.771	0.691	0.761	0.025	0.26	0.53	0.22
Week 1 to 4	0.732	0.727	0.724	0.766	0.708	0.729	0.021	0.53	0.62	0.36
BW variation, CV										
Initial	6.16	7.48	7.39	6.63	5.92	5.66	1.12	0.80	0.53	0.66
Final	15.75	13.29	13.82	11.69	11.29	12.83	2.06	0.69	0.11	0.22

[1]Pigs (n = 180; 28 d) were fed diets containing 0 to 0.4% NaB or tylosin diets containing 110 mg of tylosin/kg of complete diet. Phase 1 diets were fed wk 1 and 2 after weaning, and phase 2 diets were fed wk 3 and 4 after weaning; n = 6 pens/treatment group.
[2]*P*-value for all treatments, including the diet containing tylosin.

RESULTS

Growth Performance

Effects of adding increasing levels of sodium butyrate or tylosin (Exp. 1) on weanling pig growth performance are presented in Table 3. Addition of sodium butyrate or dietary tylosin had no effect on final BW, G:F, or BW variability. There was both a linear (R^2 = 0.11) and quadratic (R^2 = 0.16) trend ($P < 0.09$) for phase 2 ADG and a linear trend (R^2 = 0.11; $P < 0.07$) for overall ADG to decrease with increasing levels of sodium butyrate. A linear decrease (R^2 = 0.13; $P < 0.05$) was also found in phase 2 ADFI and a linear trend (R^2 = 0.13; $P < 0.06$) toward decreased overall ADFI as dietary levels of sodium butyrate were increased. The growth performance data for the second experiment, which evaluated effects of 0.2% sodium butyrate or carbadox on growth performance and effects of 0.2% sodium butyrate on the response to LPS, are presented in Table 4. Feeding diets containing 0.2% sodium butyrate or carbadox did not alter growth performance, ADFI, or BW variation when compared with pigs fed the control diets. However, during phase 1, pigs fed diets containing 0.2% sodium butyrate had decreased ($P < 0.05$) ADG and tended ($P < 0.06$) to have decreased G:F compared with animals fed diets containing carbadox.

Effect of Sodium Butyrate on Response to LPS and Carbadox on Basal Measures

Within a short time after injection, pigs challenged with LPS became visibly depressed and several pigs vomited. As expected, the LPS challenge increased ($P < 0.05$) serum concentrations of IL-6 and TNFα, and decreased ($P < 0.05$) serum IGF-I (Table 5). Pigs challenged with LPS had decreased ($P < 0.05$) serum glucose and triglyceride concentrations, but LPS had no effect on serum blood urea nitrogen, insulin, or NEFA concentrations. There was an interaction ($P < 0.05$) between dietary sodium butyrate and LPS challenge because the magnitude of the increase in serum cortisol in response to LPS was greater ($P < 0.05$) in pigs fed butyrate than in pigs fed the control diet. However, dietary butyrate had no effect on any other serum variables either in pigs injected with LPS or in pigs at the basal state. Pigs fed diets containing carbadox had lower ($P < 0.05$) basal levels of serum TNFα than pigs fed the diet containing sodium butyrate or pigs fed the control diet.

Injecting pigs with LPS decreased ($P < 0.05$) the relative abundance of IGF-I mRNA and increased ($P < 0.05$) the relative abundance of IL-6 mRNA in LM tissue (Table 6). The LPS challenge increased ($P < 0.05$) the relative abundance of both IL-6 and TNFα mRNA in liver tissue. Dietary butyrate or carbadox had no effect on the basal relative abundance of cytokine or IGF-I mRNA. However, there tended ($P < 0.10$) to be an interaction between dietary butyrate and injection with LPS for the relative abundance of IL-6 mRNA in LM tissue. In pigs challenged with LPS, the increase in LM tissue IL-6 mRNA relative abundance was greater for pigs fed sodium butyrate than for pigs fed the control diet.

DISCUSSION

A major objective of these studies was to determine the effect of increasing levels of dietary sodium butyrate on the growth performance of weanling pigs. Previous

Table 4. Effect of 0.2% dietary sodium butyrate (NaB) or carbadox on the growth performance of weanling pigs (Exp. 2)

	Treatment[1]				
Item	Control	0.2% NaB	Carbadox	SE	*P*-value
Initial BW, kg	6.3	6.3	6.3	0.4	0.98
Final BW, kg	12.7	12.5	13.4	0.5	0.37
ADG, kg					
Phase 1	0.143[ac]	0.129[ab]	0.158[c]	0.007	0.04
Phase 2	0.408	0.397	0.433	0.015	0.21
Week 1 to 4	0.266	0.256	0.284	0.011	0.20
ADFI, kg					
Phase 1	0.196	0.188	0.203	0.008	0.41
Phase 2	0.596	0.553	0.625	0.024	0.13
Week 1 to 4	0.353	0.328	0.373	0.015	0.15
G:F					
Phase 1	0.731[ac]	0.684[ab]	0.776[c]	0.025	0.06
Phase 2	0.686	0.718	0.694	0.014	0.29
Week 1 to 4	0.754	0.779	0.762	0.011	0.33
BW variation, CV					
Initial	3.16	3.21	3.25	0.46	0.99
Final	12.94	10.33	10.59	1.72	0.57

[a–c]Row values with different superscript letters are different ($P < 0.05$).
[1]Pigs (n = 108; 28 d) were fed control diets or diets containing 0.2% NaB or 55 mg of carbadox/kg of complete diet. Phase 1 diets were fed wk 1 and 2 after weaning, and phase 2 diets were fed wk 3 and 4 after weaning; n = 6 pens/treatment group.

studies evaluating effects of dietary butyrate on growth performance have found conflicting results. One study found that sodium butyrate fed to weanling pigs at a level of 0.3% increased feed efficiency (Manzanilla et al., 2006), whereas in another study, supplementing liquid-fed neonatal piglets with 0.3% sodium butyrate increased BW gain (Kotunia et al., 2004). A recently published study that evaluated dietary levels of sodium butyrate similar to those used in our study found no effect on weanling pig growth performance (Biagi et al., 2007). However, in contrast to our finding that sodium butyrate linearly decreased ADFI, Biagi et al. (2007) found no impact of dietary sodium butyrate up to 0.4% on ADFI over the 6-wk feeding study. The different ADFI responses may be due to a longer duration of feeding (4 vs. 6 wk). However, it is interesting that in broiler chickens feeding 0.4%, but not 0.2%, butyrate in the glyceride form decreased ADFI (Leeson et al., 2005), which suggests that butyrate may decrease ADFI in some cases. Taken together, these data suggest that

Table 5. Effect of sodium butyrate (NaB), carbadox, and *Escherichia coli* lipopolysaccharide (LPS) on serum cytokines, hormones, and metabolites[1]

	Basal					LPS			*P*-value[3]		
Item	Control	0.2% NaB	Carbadox	SE	*P*-value[2]	Control	NaB	SE	1	2	3
IL-6, pg/mL	10.0	10.0	10.0	—	—	2,538	4,792	893	0.22	0.0006	0.22
TNFα,[4] pg/mL	137.2[a]	141.6[a]	104.4[b]	10.2	0.04	6,377	9,527	2,257	0.49	0.003	0.49
α_1-AGP,[5] μg/mL	942	803	903	64	0.31	743	793	67	0.51	0.13	0.17
Cortisol, μg/dL	8.7	10.2	11.4	1.4	0.40	29.3[x]	90.7[y]	11.3	0.01	0.0002	0.02
Insulin, ng/mL	0.04	0.06	0.04	0.02	0.50	0.04	0.06	0.02	0.15	0.98	0.87
IGF-I, ng/mL	107.3	101.2	112.8	14.1	0.84	64.5	48.7	10.3	0.30	0.0002	0.64
Blood urea nitrogen, mg/dL	14.3	11.2	11.3	1.2	0.17	13.4	13.3	1.5	0.29	0.66	0.33
Glucose, mg/dL	84.4	79.6	77.2	3.0	0.25	72.2	70.9	3.4	0.39	0.006	0.62
NEFA, mEq/L	0.08	0.19	0.12	0.05	0.28	0.22	0.13	0.06	0.88	0.48	0.12
Triglycerides, mg/dL	71.0	64.8	62.1	7.7	0.70	44.5	42.1	6.9	0.54	0.002	0.79

[a,b]Row values with different superscripts differ ($P < 0.05$) for the basal state.
[x,y]Row values with different superscripts are different ($P < 0.05$) within the LPS treatment group.
[1]Pigs were fed control diets or diets containing 0.2% NaB or 55 mg of carbadox/kg of complete diet. After 2 wk on the experimental diets, a subset of the pigs fed control diets or diets containing NaB were injected with 25 μg/kg of BW of *E. coli* LPS. The remaining pigs that were sampled were injected with sterile saline solution. Blood samples were collected 4 h post-LPS injection; n = 6 pigs/treatment group.
[2]*P*-value for pigs in the basal state (saline injection).
[3]*P*-values for LPS challenge: 1 = dietary treatment effect; 2 = LPS effect; and 3 = interaction of diet × LPS injection.
[4]TNFα = tumor necrosis factor α.
[5]α_1-AGP = α-1-acid glycoprotein.

Table 6. Effect of sodium butyrate (NaB), carbadox, and *Escherichia coli* lipopolysaccharide (LPS) on the relative abundance of selected mRNA[1]

	Basal					LPS			*P*-value[3]		
Item	Control	0.2% NaB	Carbadox	SE	*P*-value[2]	Control	NaB	SE	1	2	3
LM mRNA											
IGF-I	0.017	0.027	0.018	0.006	0.37	0.007	0.005	0.005	0.32	0.003	0.21
IL-6	0.002	0.003	0.004	0.001	0.70	0.043[x]	0.238[y]	0.054	0.09	0.02	0.10
TNFα[4]	0.015	0.029	0.016	0.005	0.13	0.023	0.044	0.011	0.15	0.35	0.77
Liver mRNA											
IGF-I	0.038	0.021	0.018	0.007	0.14	0.017	0.037	0.010	0.92	0.78	0.28
IL-6	0.004	0.003	0.005	0.001	0.21	0.149	0.228	0.086	0.66	0.05	0.66
TNFα	0.074	0.054	0.065	0.016	0.67	0.365	0.352	0.063	0.80	0.002	0.95
Ileum mRNA											
IGF-I	0.054	0.012	0.037	0.020	0.35	0.018	0.014	0.014	0.12	0.25	0.20
IL-6	0.016	0.009	0.018	0.005	0.52	0.018	0.017	0.006	0.49	0.44	0.62
TNFα	0.22	0.10	0.12	0.06	0.35	0.08	0.07	0.054	0.23	0.14	0.32

[x,y]Row means with different superscripts are different ($P < 0.05$) within the LPS treatment group.
[1]Pigs were fed control diets or diets containing 0.2% NaB or 55 mg of carbadox/kg of complete diet. After 2 wk on the experimental diets, a subset of the pigs fed control diets or diets containing NaB were injected with 25 μg/kg of BW of *E. coli* LPS. The remaining pigs that were sampled were injected with sterile saline solution. Muscle, liver, and ileum tissue samples were collected 4 h post-LPS injection; n = 6 pigs/treatment group. The cytokine and IGF-I mRNA are normalized to the expression levels of cyclophilin A.
[2]*P*-value for pigs in the basal state (saline injection).
[3]*P*-values for LPS challenge: 1 dietary treatment effect; 2 LPS effect; and 3 interaction of diet × LPS injection.
[4]TNFα = tumor necrosis factor α.

the response to dietary butyrate is variable, and may depend on pig age, feeding duration, and diet type.

A previous study conducted in young pigs found that parenterally fed short-chain fatty acids increased intestinal IL-6 abundance (Milo et al., 2002). The lack of an effect on the intestinal relative abundance of cytokine mRNA found in our experiment may be due to the different routes of administration for the butyrate, or that Milo et al. (2002) fed a combination of short-chain fatty acids. Butyrate can be absorbed rapidly through stomach tissue (Bugaut, 1987), and thus may not arrive intact to the lower digestive tract to affect intestinal cytokine abundance. Indeed, a recent study in young pigs fed 0.3% sodium butyrate found that increased butyrate levels were present in the stomach, but not in the jejunum, of the pigs fed sodium butyrate (Manzanilla et al., 2006). Results of 2 additional recent studies indicate that sodium butyrate may be absorbed in the upper gastrointestinal tract and therefore may not directly affect the intestine. Claus et al. (2006) found that feeding growing pigs a fat-coated butyrate product increased the small intestinal plica area, whereas Biagi et al. (2007) found no effect of feeding sodium butyrate on intestinal morphology. Feeding fermentable fiber to pigs in the form of rye bread increased peripheral plasma butyrate concentrations (Bach Knudsen et al., 2005). Therefore, dietary butyrate or diet-derived butyrate may be absorbed from the digestive tract and may be present in peripheral circulation, which may lead to systemic effects for dietary butyrate.

The increase in cortisol in LPS-challenged pigs was greater in pigs fed 0.2% sodium butyrate. This is not the first report of a dietary ingredient increasing the cortisol response to LPS in weanling pigs. Touchette et al. (2002) found that pigs fed spray-dried plasma had a greater increase in serum cortisol in response to LPS. However, spray-dried plasma also increased serum TNFα and interferon-γ, suggesting that feeding plasma increased the magnitude of the acute phase response. In our study, dietary butyrate did not exacerbate the increase in serum TNFα as it did for cortisol. This suggests different mechanisms for spray-dried plasma and butyrate to increase the magnitude of the LPS-induced surge in serum cortisol. Studies in sheep have found that ruminal infusion of a mixture of short-chain fatty acids (acetic, propionate, and butyrate) increases plasma cortisol (Boukhliq and Martin, 1997). However, it is unknown whether butyrate infusion alone would elevate cortisol levels.

In the current study, we found no effect of butyrate on tissue IGF-I mRNA abundance or serum IGF-I. The lack of an effect on IGF-I found in the current study may be related to the finding that there were no effects of dietary butyrate on ADG or G:F. It was initially hypothesized that because of the previously found anti-inflammatory activity of butyrate (Zhang et al., 2007) and other histone deacetylase inhibitors (Leoni et al., 2005), butyrate may decrease the cytokine response to LPS, and perhaps blunt the inflammation-induced decrease in muscle and serum IGF-I (Spurlock et al., 1998). Manzanilla et al. (2006) suggested that the increase in G:F found in pigs fed butyrate may be due to increased activation of the IGF-I axis. Indeed, studies in which antibiotics have been found to increase G:F have found an increase in plasma IGF-I (Hathaway et al., 1996, 1999). Nonetheless, results of our experiment confirm that LPS challenge increases muscle cytokine expression in the pig, as found in rodents (Frost et al., 2002; Lang et al., 2003). We are aware of only one other report in pigs (Brix-Cristensen et al., 2005) that

measured increased skeletal muscle cytokine expression in response to LPS.

Ours is the first study to find that butyrate may augment cytokine expression in muscle after an inflammatory challenge, and extends to skeletal muscle the proinflammatory effects of butyrate that are sometimes found. Infusion (Haddad et al., 2005), injection (Janssen et al., 2005), or overexpression (Lieskovska et al., 2003) of IL-6 in rodent models has been found to lead to skeletal muscle atrophy. Broilers fed 0.2% butyrate glycerides were found to have a 7% greater yield of breast muscle (Leeson et al., 2005), suggesting that dietary butyrate appears to be anabolic in nonchallenged animals. However, in our study no anabolic results were found in terms of somatic growth or IGF-I expression. Whether the increase in muscle yield in broilers was indirectly mediated by butyrate altering cytokine production or was a direct effect of butyrate on myoblasts remains undetermined. However, Iezzi et al. (2002) found that butyrate can act directly on myoblasts to stimulate myogenesis.

A growth-promoting response to either dietary tylosin or carbadox was not found in the current study. The lack of response in the current study could be due to diet complexity because all diets fed contained animal plasma. Feeding weanling pigs diets containing spray-dried plasma increased growth performance and decreased steady-state cytokine expression and presumed immune system activation (Touchette et al., 2002). The lack of response to antibiotics could also be due to a low incidence of pathogen exposure and the fact that the pigs were of a high health status. There were no pig mortalities in the current study, nor was serum AGP affected by dietary treatment. Previous studies in weanling pigs have shown a relationship between presumed pathogen load and serum AGP concentrations (Williams et al., 1997). A recently published study (Walsh et al., 2007) also found no growth-promoting response to carbadox, which the authors attributed to the high health status of the pigs. On the basis of the lack of difference in overall growth performance, it seems unlikely that there were differences in pathogen loads between pigs fed the control diet, sodium butyrate, or carbadox in the current study.

Serum TNFα concentrations were slightly decreased in pigs fed carbadox compared with pigs fed the butyrate or control diets. There is an emerging hypothesis that one mechanism by which antibiotics increase growth performance is through a direct reduction in inflammation and its associated catabolic events. Several classes of antibiotics have been found to inhibit the inflammatory activity of phagocytic cells directly (Niewold, 2007). The decrease in serum TNFα was not associated with increased growth performance in pigs fed carbadox, nor was it associated with increased tissue or serum IGF-I. Perhaps increased levels of immune system activation, as would be indicated by higher levels of cytokines and AGP, are required for carbadox to increase growth and IGF-I.

In conclusion, feeding weanling pigs sodium butyrate did not enhance growth performance or feed efficiency but did linearly decrease ADFI. Likewise, feeding the dietary antibiotics tylosin or carbadox had no impact on growth performance when compared with pigs fed control diets. Pigs fed carbadox had decreased serum TNFα concentrations, but carbadox had no impact on steady-state cytokine or IGF-I mRNA expression. In pigs challenged with LPS, sodium butyrate increased the magnitude of the cortisol response and further increased skeletal muscle IL-6 mRNA expression. These data suggest that dietary sodium butyrate affects the response to inflammatory stimuli, but further research is necessary to determine the implications for pig growth when pigs are subjected to inflammatory stimuli.

LITERATURE CITED

Bach Knudsen, K. E., A. Serena, A. Kirstin, B. Kjaer, H. Jorgensen, and R. Engberg. 2005. Rye bread enhances the production and plasma concentration of butyrate but not the plasma concentrations of glucose and insulin in pigs. J. Nutr. 135:1696–1704.

Biagi, G., A. Piva, M. Moschini, E. Vezzali, and F. X. Roth. 2007. Performance, intestinal microflora, and wall morphology of weanling pigs fed sodium butyrate. J. Anim. Sci. 85:1184–1191.

Boukhliq, R., and G. B. Martin. 1997. Administration of fatty acids and gonadotrophin secretion in the mature ram. Anim. Reprod. Sci. 49:143–159.

Brix-Christensen, V., J. Gjedsted, S. K. Andersen, C. Vestergaard, J. Nielsen, T. Rix, R. Nyboe, N. T. Anderson, A. Larsson, O. Schmitz, and E. Tønnesen. 2005. Inflammatory response during hyperglycemia and hyperinsulinemia in a porcine endotoxemic model: The contribution of essential organs. Acta Anaesthesiol. Scand. 49:991–998.

Bugaut, M. 1987. Occurrence, absorption, and metabolism of short chain fatty acids in the digestive tract of mammals. Comp. Biochem. Physiol. 86:439–472.

Claus, R., D. Gunthner, and H. Letzgub. 2006. Effects of feeding fat-coated butyrate on mucosal morphology and function in the small intestine of the pig. J. Anim. Physiol. Anim. Nutr. (Berl.) 91:312–318.

Frost, R. A., G. J. Nystrom, and C. H. Lang. 2002. Lipopolysaccharide regulates proinflammatory cytokine expression in mouse myoblasts and skeletal muscle. Am. J. Physiol. 283:R698–R709.

Fukae, J., Y. Amasaki, T. Bohgaki, S. Yasuda, S. Jodo, T. Atsumi, and T. Koike. 2005. Butyrate suppresses tumor necrosis factor α production by regulating specific messenger RNA degradation mediated through a *cis*-acting AU-rich element. Arthritis Rheum. 52:2697–2707.

Haddad, F., F. Zaldivar, D. M. Cooper, and G. R. Adams. 2005. IL-6-induced skeletal muscle atrophy. J. Appl. Physiol. 98:911–917.

Hathaway, M. R., W. R. Dayton, M. E. White, T. L. Henderson, and T. B. Henningson. 1996. Serum insulin-like growth factor I (IGF-I) concentrations are increased in pigs fed antimicrobials. J. Anim. Sci. 74:1541–1547.

Hathaway, M. R., W. R. Dayton, M. E. White, T. L. Henderson, D. A. Young, and T. N. Doan. 1999. Effect of feed intake on antimicrobially induced increases in porcine serum insulin-like growth factor I. J. Anim. Sci. 77:3208–3214.

Iezzi, S., G. Cossu, C. Nervi, V. Sartorelli, and P. L. Puri. 2002. Stage-specific modulation of skeletal myogenesis by inhibitors of nuclear deacetylases. Proc. Natl. Acad. Sci. USA 99:7757–7762.

Janssen, S. P., G. Gayan-Ramirez, A. Van den Bergh, P. Herijgers, K. Maes, E. Verbeken, and M. Decramer. 2005. Interleukin-6 causes myocardial failure and skeletal muscle atrophy in rats. J. Anim. Sci. 111:996–1005.

Kerr, B. J., M. T. Kidd, J. A. Cuaron, K. L. Bryant, T. M. Parr, C. V. Maxwell, and J. M. Campbell. 2004. Isoleucine requirements and ratios in starting (7 to 11 kg) pigs. J. Anim. Sci. 82:2333–2342.

Kotunia, A., J. Wolinski, D. Laubitz, M. Jurkowska, V. Rome, P. Guilloteau, and R. Zabielski. 2004. Effect of sodium butyrate on the small intestine development in neonatal piglets fed by artificial sow. J. Physiol. Pharmacol. 55:59–68.

Lang, C. H., C. Silvis, N. Deshpande, G. Nystrom, and R. A. Frost. 2003. Endotoxin stimulates in vivo expression of inflammatory cytokines tumor necrosis factor alpha, interleukin-1β, -6, and high-mobility group protein-1 in skeletal muscle. Shock 19:538–546.

Leeson, S., H. Namkung, M. Antongiovanni, and E. H. Lee. 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poult. Sci. 84:1418–1422.

Leoni, F., G. Fossati, E. C. Lewis, J. K. Lee, G. Porro, P. Pagani, D. Modena, M. L. Moras, P. Pozzi, L. L. Reznikov, B. Siegmund, G. Fantuzzi, C. A. Dinarello, and P. Mascagni. 2005. The histone deacetylase inhibitor ITF2357 reduces production of pro-inflammatory cytokines in vitro and systemic inflammation in vivo. Mol. Med. 11:1–15.

Li, C. J., and T. H. Elsasser. 2005. Butyrate-induced apoptosis and cell cycle arrest in bovine kidney epithelial cells: Involvement of caspase and proteosome pathways. J. Anim. Sci. 83:89–97.

Lieskovska, J., D. Guo, and E. Derman. 2003. Growth impairment in IL-6-overexpressing transgenic mice is associated with induction of SOCS3 mRNA. Growth Horm. IGF Res. 13:26–35.

Manzanilla, E. G., M. Nofrarias, M. Anguita, M. Castillo, J. F. Perez, S. M. Matin-Orue, C. Kamel, and J. Gasa. 2006. Effects of butyrate, avilamycin, and a plant extract combination on the intestinal equilibrium of early-weaned pigs. J. Anim. Sci. 84:2743–2751.

Milo, L. A., K. A. Reardon, and K. A. Tappenden. 2002. Effects of short-chain fatty acid-supplemented total parenteral nutrition on intestinal pro-inflammatory cytokine abundance. Dig. Dis. Sci. 47:2049–2055.

Niewold, T. A. 2007. The nonantibiotic, anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult. Sci. 86:605–609.

NRC. 1998. Nutrient Requirements of Swine. 10th ed. Natl. Acad. Press, Washington, DC.

Rodriguez-Cabezas, M. E., J. Galvez, D. Camuesco, M. D. Lorente, A. Concha, O. Martinez-Augustin, L. Redondo, and A. Zarzuelo. 2003. Intestinal anti-inflammatory activity of dietary fiber (*Plantago ovata* seeds) in HLA-B27 transgenic rats. Clin. Nutr. 22:463–471.

Schroeder, F. A., C. L. Lin, W. E. Crusio, and S. Akbarian. 2007. Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. Biol. Psychiatry 62:55–64.

Spurlock, M. E., M. A. Ranalletta, S. G. Cornelius, G. R. Frank, G. M. Willis, S. Ji, A. L. Grant, and C. A. Bidwell. 1998. Leptin expression in porcine adipose tissue is not increased by endotoxin but is reduced by growth hormone. J. Interferon Cytokine Res. 18:1051–1058.

Touchette, K. J., J. A. Carroll, G. L. Allee, R. L. Matteri, C. J. Dyer, L. A. Beausang, and M. E. Zannelli. 2002. Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs. J. Anim. Sci. 80:494–501.

Walsh, M. C., D. M. Sholly, R. B. Hinson, S. A. Trapp, A. L. Sutton, J. S. Radcliffe, J. W. Smith II, and B. T. Richert. 2007. Effects of Acid LAC and Kem-Gest acid blends on growth performance and microbial shedding in weanling pigs. J. Anim. Sci. 85:459–467.

Weber, T. E., and M. E. Spurlock. 2004. Leptin alters antibody isotype in the pig in vivo, but does not regulate cytokine expression or stimulate STAT3 signaling in peripheral blood monocytes in vitro. J. Anim. Sci. 82:1630–1640.

Williams, N. H., T. S. Stahly, and D. R. Zimmerman. 1997. Effect of chronic immune system activation on the rate, efficiency, and composition of growth and lysine needs of pigs fed from 6 to 27 kg. J. Anim. Sci. 75:2463–2471.

Wright, K. J., R. Balaji, C. M. Hill, S. S. Dritz, E. L. Knoppel, and J. E. Minton. 2000. Integrated adrenal, somatotropic, and immune responses of growing pigs to treatment with lipopolysaccharide. J. Anim. Sci. 78:1892–1899.

Zhang, L. T., Y. M. Yao, J. Q. Lu, X. J. Yan, Y. Yu, and Z. Y. Sheng. 2007. Sodium butyrate prevents lethality of severe sepsis in rats. Shock 27:672–677.